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A Gustotopic Map of Taste Qualities in the Mammalian Brain

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Abstract

The taste system is one of our fundamental senses, responsible for detecting and responding to sweet, bitter, umami, salty and sour stimuli. In the tongue, the five basic tastes are mediated by separate classes of taste receptor cells each finely tuned to a single taste quality. Here, we explored the logic of taste coding in the brain by examining how sweet, bitter, umami and saltiness are represented in the primary taste cortex. Using in vivo two-photon calcium-imaging we demonstrated striking topographic segregation in the functional architecture of the gustatory cortex. Each taste quality is represented in its own separate cortical field, revealing the existence of a gustotopic map in the brain. These results expose the basic logic for the central representation of taste.

The sense of taste is in charge of evaluating the nutritional value of a meal. In mammals, a very small palette of just five taste qualities orchestrates appetitive responses to energy- and protein-rich food sources (sweet and umami), and warns against the ingestion of toxic or spoiled/fermented foods (bitter and sour/carbonated). Salt sensing aids the adequate consumption of sodium, while cautioning against the ingestion of excess salt. Notably, the attraction towards sweet, umami and low-sodium, and the aversion towards bitter, sour and high salt is innate and largely invariant throughout life. These observations suggest a physiological hard wiring of tastant quality to hedonic value.

Each of the 5 basic tastes is detected by specialized sensors expressed on receptor cells of the tongue and palate epithelium. Sweet and umami are mediated by a small family of 3 GPCRs that combine to form two heteromeric receptors, T1R1+3 for umami (1–3) and T1R2+3 for all sweet-tasting compounds (1, 3, 4). Bitters are detected by a large family of GPCRs, the T2Rs, that respond to the complex and diverse universe of bitter tastants (5–8); sour and sodium taste are sensed by ion channel receptors (9–11). Over the past 10 years, we have shown that each receptor class is expressed in its own distinct taste cell type (2–6, 8, 12, 13). This one-taste/one-cell-class coding scheme has emerged as the hallmark of the organization of the mammalian taste system at the periphery, and the mechanism through which individual taste qualities are recognized and encoded in the tongue (9, 14).

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In rodents, information from taste cells in the oral cavity is transmitted to the primary taste cortex, the insula, through multiple neural stations (14, 15). How are the chemical senses represented in the cortex? Recent studies on the representation of odors in the primary olfactory cortex revealed a sparse and distributed pattern of neuronal activity whereby each odor is encoded by a unique ensemble of neurons, but without any spatial clustering or preference in relation to odorant space (16). This is in contrast to the organization of primary visual, somatic and auditory cortex, where neurons that respond to similar features of the sensory stimulus are topographically organized into spatial maps in the cortex (17–19).

Previous studies examining how tastes are represented in the primary gustatory cortex have relied on a number of elegant approaches, including imaging of intrinsic signals (20, 21), tracer labeling techniques [WGA (22)], and electrophysiological recordings from individual cells [or a handful of cells at the time (23–25)]. Unfortunately, these experiments have led to inconclusive, and often contradictory views of the central representation of taste, in part due to the limited spatial resolution of the techniques, and/or the shortfalls of recording from small numbers of neurons (15, 26). We reasoned that if one could simultaneously examine the activity of large numbers of neurons in insular cortex in response to taste stimulation (27), and do so with single cell resolution, it should be possible to determine how tastes are represented in the primary taste cortex.

Imaging gustatory responses in primary taste cortex

We used two-photon calcium imaging (16, 28–30) to monitor tastant-evoked neural activity across the gustatory cortex in vivo. To confirm the appropriate region for imaging in mice (20, 25), we used extracellular electrodes to record tastant-evoked responses in the gustatory area of the thalamus (Fig. 1), and after identifying taste-responding cells, we used an AAV2/hu11-GFP virus (31) as an anterograde tracer to label (and trace) projections to primary taste cortex (Fig. 1c; see Methods). These studies demarcated a domain of approximately 2.5 mm^2 in the insula, located ~ 1 mm above the intersection of the middle cerebral artery (MCA, Fig.1a,c) and the rhinal veins, and extending ~ 1 mm anteriorly, 1.5 mm posteriorly and 1 mm dorsoventrally. Therefore, small areas of cortex were exposed in this region of the insula of anesthetized animals (via surgical craniotomy), and the neurons bulk-loaded with the calcium sensitive dye Oregon Green 488 BAPTA-1 AM (OGB-AM) for functional imaging (Fig. 1d); this dye is effectively taken up by the cells (Fig. S1), and serves as a robust fluorescent sensor of neural activity with high signal to noise ratios (16, 29, 30). To ensure that responses reflect biologically relevant stimuli, we used concentrations of tastants that reliably elicit ~80% of the maximal response in behavioral studies (3, 8).

Representation of bitter taste

We systematically inspected layer 2/3 of gustatory cortex (a layer readily accessible by 2-photon imaging) for tastant-evoked responses by parsing and tiling the insula into fields of 350 × 350 μm and testing for neurons exhibiting correlated patterns of tastant-dependent firing (16). The stimulus paradigm consisted of a 30 s pre-stimulus application of artificial saliva (AS), a 10 s exposure to a test tastant, and a 30 s AS post-stimulus wash (see Methods for details). We began by focusing on bitter taste, and discovered a highly localized, topographically defined cluster (i.e. a “hot spot” at ~1 mm dorsal to the rhinal veins and ~1 mm posterior to the MCA, see Methods for details), where many neurons responded to bitter stimuli with highly significant increases in intracellular calcium ($\Delta F/F$ ranging between 20–65%; Fig. 2); the location of the hot spot was stereotyped so that we could consistently find this cortical field in multiple animals (Fig. 2f and Fig. S2). We examined the reproducibility of the taste-evoked responses in the hot spot by measuring variability across trials. We imaged approximately 200 neurons per trial, and these were segmented and coded according

to their location and response magnitude (16, 32). On average, approximately one third of the OGB-labeled neurons fired during the window of bitter tastant application, and the vast majority of these (> 70%) responded in multiple bitter-stimulation trials (compare Fig. 2a and 2b, see also Fig. S3). By examining bitter responses across the entire gustatory cortex in multiple animals, we confirmed this hot spot (i.e. cortical field), as the only region that exhibited correlated responses to bitter stimuli (Fig. 2f and Fig. S2).

Are the bitter-responsive neurons selective or broadly tuned? Our approach was to record from the bitter hot spot while stimulating the tongue with tastants representing the different taste qualities. Bitter-responding neurons were exquisitely tuned to bitter taste (Fig. 2), with highly significant preference for bitter versus any other taste quality. No other taste qualities were represented in this spatial domain of gustatory cortex (Fig. 2). Equivalent results were obtained in single unit electrophysiological recordings from within the bitter hot spot (see Fig S4).

Humans and rodents recognize a wide range of bitter tasting chemicals, and correspondingly express a large number of bitter taste receptor (T2R) genes (5–7). Most, if not all, T2Rs are expressed in the same subset of taste receptor cells (5), implying that these cells act as broadly tuned bitter sensors, capable of detecting a wide range of bitter tasting chemicals (5, 8). Thus, we predicted that the cortical representation for different bitter tastants should be shared. Therefore, we assayed the responses to several chemically distinct bitter tastants, and indeed all of them activated the same hot spot (Fig. 3 and Fig. S3).

Specificity of cortical responses to taste stimuli

To further validate the specificity of the bitter cortical field we examined taste responses in engineered mice where a single one of the bitter taste receptors had been eliminated. T2R5 is one of the 36 bitter receptors in the mouse genome (33); it is necessary (and sufficient) for physiological and behavioral taste responses to the toxin cycloheximide, but not for detecting a wide range of other bitter chemicals (6, 8). We reasoned that if the bitter-evoked activity in the hot spot is indeed used to represent bitter taste, then all responses to cycloheximide should be missing in a T2R5 knockout animal. However, responses to other bitter tastants should remain. This selective-loss experiment alleviates the uncertainty associated with examining animals with a total loss of bitter taste [e.g. TRPM5 or PLCβ2 knockouts (13)], as such a negative result could not be distinguished from a trivial failure to record cortical responses. Indeed, as predicted, cycloheximide responses were abolished in the bitter hotspot of T2R5-KO animals, but responses to other bitter chemicals were indistinguishable from those in control mice (Fig. 3). Taken together, these studies demonstrate topographic gustatory selectivity in the organization of the primary taste cortex, and substantiate this hot spot as a selective cortical representation of bitter taste in the insula.

A spatial map of taste qualities

Given the existence of a topographically defined region for the representation of bitter taste, we hypothesized that sweet would also be encoded in its own cortical field, and therefore surveyed the insular cortex for selective activity to sweet tastants. We discovered such a specific sweet hot spot at a considerable distance from the bitter hot spot (approximately 2.5 mm rostro-dorsal to the bitter field; Fig. 4a–b). There, a large ensemble of neurons responded to sweet taste stimulation of the tongue. Multiple lines of evidence validate that area as the sweet cortical field: First, the cells were preferentially tuned to sweet versus any of the other taste qualities (Fig 4b, Fig. S5). Second, the very same hot spot was activated by structurally different sweet-tasting chemicals (e.g. artificial versus natural sweeteners), but not by tastants for other taste qualities (Fig. 4a, b and Fig. S5). Third, responses were abolished in sweet-receptor knockout animals [T1R2-KO (3); Fig. S6 and data not shown].

Finally, this is the only region of gustatory insula that showed correlated activity in response to sweet taste.

The topographic separation of sweet and bitter taste into distinct and non-overlapping gustatory fields in primary taste cortex reveals the existence of a functional "gustotopic map"; this is very different from what is seen in the other chemosensory modality, olfaction, where individual odorants are represented in primary olfactory cortex by the activation of spatially dispersed ensembles of neurons, without any discernible topographic or chemotopic organization (16). Unlike the olfactory system, however, which must recognize, distinguish, and often initiate distinct physiological and behavioral responses to a vast universe of chemically distinct odorants, the chemical-perceptual space to be represented by the five basic taste qualities is remarkably limited (9, 14). Sweet and bitter exemplify the two poles of the gustatory spectrum, thus we wondered if the other taste qualities were also part of a gustatory map in the insula.

Umami is the savory taste humans associate with monosodium glutamate (MSG), but that in most other mammalian species is used to recognize and mediate appetitive responses to protein-rich food sources [e.g. the taste of all amino acids (1, 2, 34)]. We searched insular cortex for umami-evoked neuronal activity by stimulating the tongue with monopotassium glutamate (35). Indeed, just as seen for sweet and bitter taste, the cortical representation of umami was also part of the gustotopic map, with a unique hot spot for umami taste (Fig. 4c, d and Fig. S5). The umami cortical field was specifically tuned to umami versus the other four taste qualities. As a further proof of appropriate specificity in the umami cortical field, we also showed that this hot spot was activated by other L-amino acids (e.g. umami agonists; Fig 4d) but not by their enantiomeric inactive D-forms (2, 3).

The remaining two basic tastes, salt (sodium) and sour (acid) sensing are mediated by ion channel receptors, rather than G-protein coupled receptors (10–12, 36). Does the same organization apply to ionic tastes? Our efforts to uncover an acid (sour) hot spot did not succeed. We believe this could be because its location was outside the area sampled in our studies, or even because acid stimuli also acts on a number of other pathways, like pain and somatosensation (37), possibly leading to changes in its cortical representation. Thus, we focused on sodium taste.

Recently, we showed that low concentrations of NaCl (i.e. appetitive salt taste) are sensed by a unique population of taste receptor cells via the ENaC ion channel (12). A salient feature of this response is its inhibition by amiloride, and its strong selectivity for sodium versus other salts (12). Therefore, we searched for a salt-responsive cortical cluster that met those criteria. Just as shown for the other basic taste qualities, sodium taste is also represented in its own spatially segregated field (Fig. 4e, f and Fig. S5). The responding neurons are exquisitely tuned to NaCl versus the other taste qualities, are sensitive to amiloride, and are specific for sodium versus other cations (e.g. KCl, $MgCl_2$).

Concluding Remarks

For many years, the prevailing views for the organization and function of the taste system at the periphery centered around the concept of taste coding via broadly tuned taste receptor cells (38–40). Hence, individual taste receptor cells were proposed to express receptors for various taste qualities and thus respond to multiple taste stimuli. Now, however, we know that each of the five basic tastes is mediated by its own class of taste receptor cells, each tuned to a single taste quality (i.e. sweet-cells, bitter-cells, etc), thus defining a "one-cell one-taste" coding logic (9, 14). Notably, existing models of taste coding in insula included proposals of broadly tuned neurons across taste qualities [with no spatial segregation (15)],

as well as others suggesting a certain degree of topographic organization, but with no region dedicated to the processing of only one taste quality (20, 25, 26). While we cannot rule out the existence of sparse numbers of broadly tuned cells (24, 26) distributed throughout the taste cortex (i.e. non-clustered), our results demonstrate that the individual basic tastes are represented in the insula by finely tuned cells organized in a precise and spatially ordered gustotopic map, where each taste quality is encoded in its own (segregated) stereotypical cortical field.

The organization of the primary taste cortex appears, at first glance, reminiscent of the somatosensory, auditory, and visual system, which exhibit spatially organized somatotopic, tonotopic and retinotopic cortical maps (17–19). However, unlike these three sensory modalities where their cortical maps reflect smooth transitions across features of sensory space, this is not the case in the taste system. Furthermore, in the somatosensory, auditory and visual systems the peripheral receptors display exacting spatial order, while in taste, a distributed ensemble of peripheral receptor cells in the tongue, without any spatial organization (but with defined identities), nonetheless converge into a fixed cortical map where neurons with similar response profiles are clustered. Why should taste be represented in a spatial map? We speculate this organization has an ancient evolutionary, possibly developmental rather than a strictly functional origin. Indeed, the perceptual space to be represented by the taste system is limited to just a handful of qualities, thus having each basic taste represented in individually segregated fields provides a simple and elegant architectural solution to pattern, wire, and interconnect the ensembles of neurons representing each taste quality.

Our studies suggest that the cortical fields representing the individual basic tastes cover only a small fraction of the insula. What does the rest of the insula do? In our systematic exploration of insula we found that outside the hot spots, only small numbers of sparsely distributed neurons exhibited significant fluorescent changes during the window of tastant presentation, (Fig. S6; see also Fig. S4). Importantly, the same results were observed in animals lacking taste receptor function (e.g. in knockouts of receptor proteins; see Fig. S6), and irrespective of the quality of the taste stimulus (including to artificial saliva alone), implying these do not represent responses to the individual basic tastes. Hence, the inter hot-spot regions might be involved in other aspects of taste coding, like in the representation of taste mixes and thus help code the perception of “flavor” [e.g. responding to several rather than a single taste (24, 26, 41)]. In addition, insular cortex responds to more than just taste, and it is often thought of as a site for multisensory integration (15, 41, 42). Thus, these areas may participate in the integration of taste with the other senses.

The discovery of a gustotopic map in the mammalian cortex, together with the advent of sophisticated genetic and optical tools (43) should now make it possible to experimentally manipulate taste cortex with exquisite finesse. In future studies, it will also be important to elucidate how taste intensity is encoded in the insular cortex, and to determine whether taste qualities with similar valence project to common targets. Likewise, tracing the connectivity of each of the basic taste qualities to higher brain stations will help decipher how these integrate with other modalities, and combine with the internal and emotional states to ultimately choreograph taste behaviors (44).

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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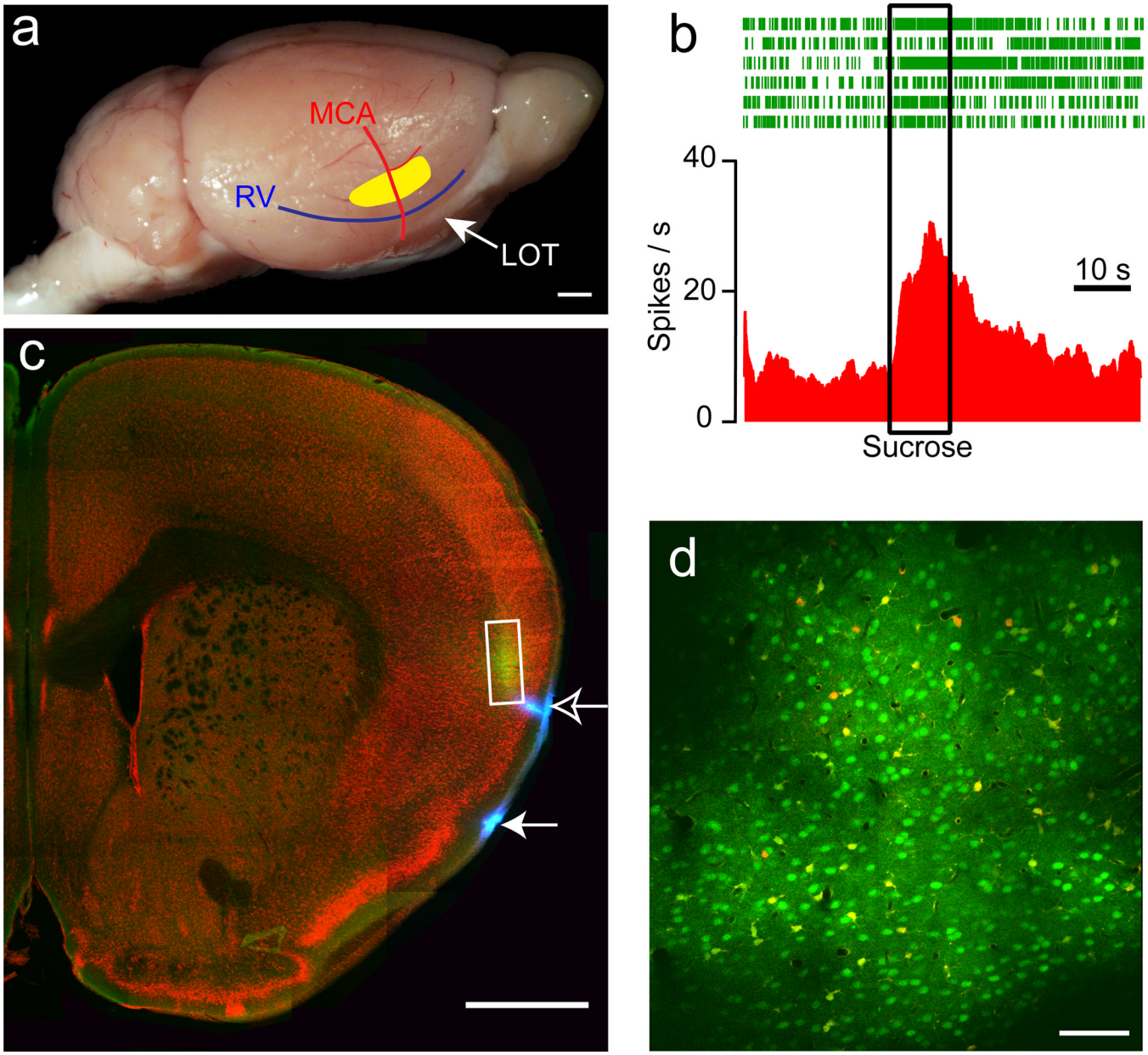

Fig. 1. Two-photon imaging in the mouse insular cortex

(a) Photograph of a mouse brain highlighting the approximate location of our imaging studies in the primary taste cortex (yellow). Also shown are the superimposed drawings of two key vascular landmarks (MCA, middle cerebral artery; RV, rhinal vein; LOT, lateral olfactory tract). (b) Responses of a sweet-sensitive thalamic taste neuron to 300 mM sucrose; the box indicates the time and duration of the sweet stimulus. After identifying such taste responsive neurons, cells around the recording site were infected with an AAV2/hu11-GFP virus to label their terminal fields in layer 4 of gustatory cortex (panel c). (c) coronal section of a mouse brain (Bregma +1.0) stained with TO-PRO-3 (red). Shown is the location of the thalamocortical projections labeled after infection with the AAV2/hu11-GFP virus (white box). In order to triangulate this region in relation to the vascular landmarks, we injected DiI at the intersection between the RV and the MCA (pseudocolored in blue; solid arrow), and at 1 mm above (open arrow). (d) Images of bulk-loaded neurons and astrocytes

in layer 2/3 of the primary gustatory cortex (see also Fig. S1) with Oregon Green 488 BAPTA-1 AM (green fluorescence) and sulforhodamine 101 (yellow labeled astrocytes). Animals were imaged *in vivo* after surgical craniotomy using two-photon microscopy. Scales: panel a, 1 mm, panel c, 1 mm; panel d, 100 μm.

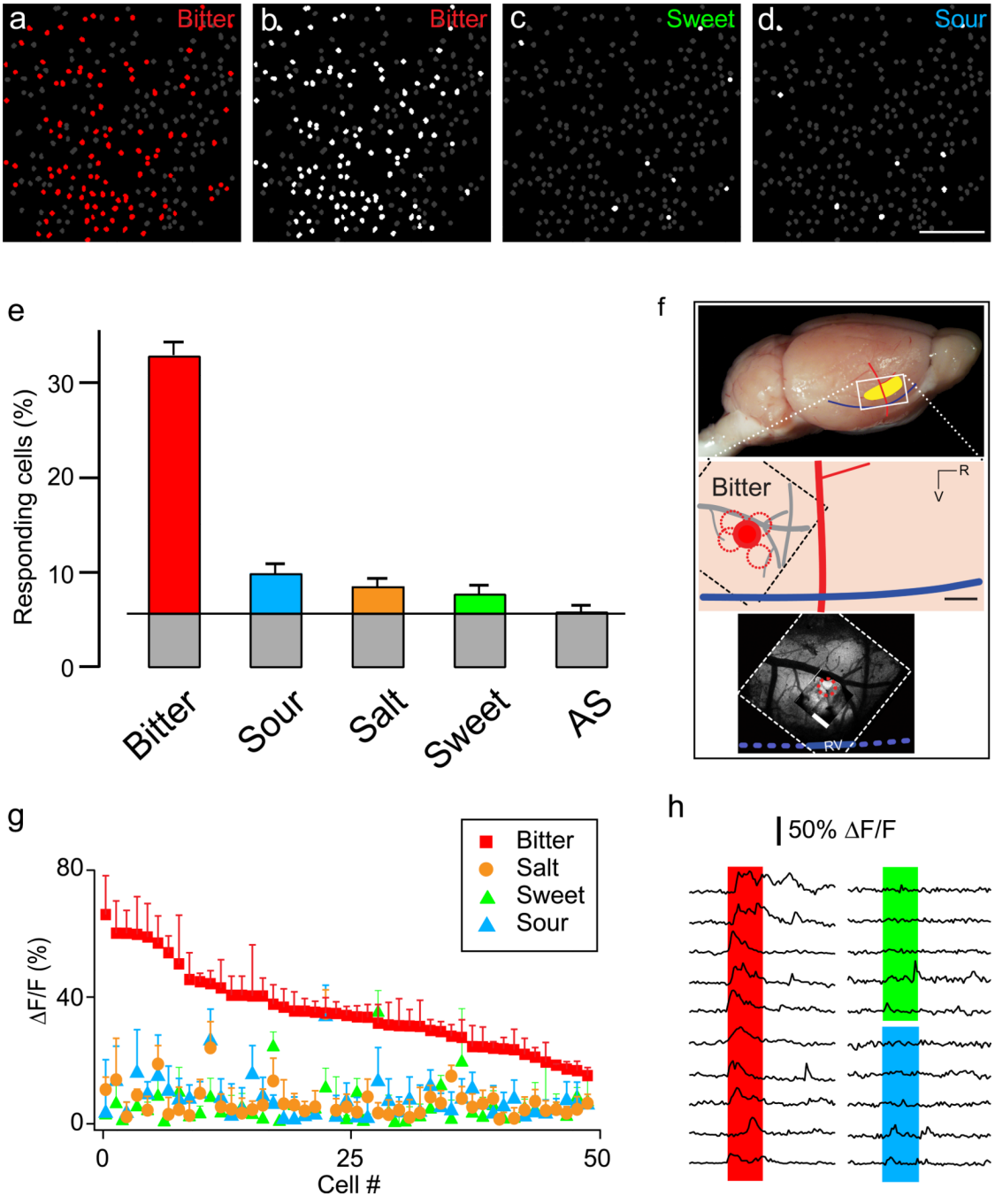

Fig. 2. Tastant-evoked responses in the bitter hot spot

(a) Bitter tastant stimulation of the tongue (single trial) evoked robust responses in the bitter hot spot; shown is an image illustrating changes in OGB-fluorescence to a 1 mM cycloheximide stimulus; approximately 35% of the loaded neurons responded with ΔF/F greater than 3.5 standard deviations above background (see also Fig. S7). (b–d). In sharp contrast to the sparse activity seen during application of other tastants (panels c and d; see text), neurons activated by bitter responded over multiple trials; cells that responded in at least 2 of 4 trials are labeled white (panel b). (e) Neurons in the bitter hot spot responded selectively to bitter but not to other taste stimuli (see methods for details) (n = 8). (f) Also shown is an illustration, and a bright-field image, depicting the approximate relation of the

bitter cortical field (red circle) to the vascular landmarks; the dotted circles depict the location of the bitter hot spot in 4 additional animals (see Methods for details). The middle panel has been flattened to present a 2-dimensional view of this area of the brain. (g) Bitter-responsive neurons are highly tuned to bitter taste (red). The graph shows the rank-ordered $\Delta F/F$ of a set of bitter-responsive neurons in a six trial experiment to bitter stimuli versus other tastants; bitter = 1 mM cycloheximide, NaCl = 100 mM NaCl, sweet = 30 mM acesulfame K, sour = 10 mM citric acid. There was no apparent organization based on response amplitudes within the cluster (Fig. S8). (h) Representative OGB-fluorescence changes during a 10 s bitter (red), sweet (green), and sour (blue) stimulation. Scales (panels a–d) 100 μm, and (f) 0.5 mm. Error bars are mean ± s.e.m.

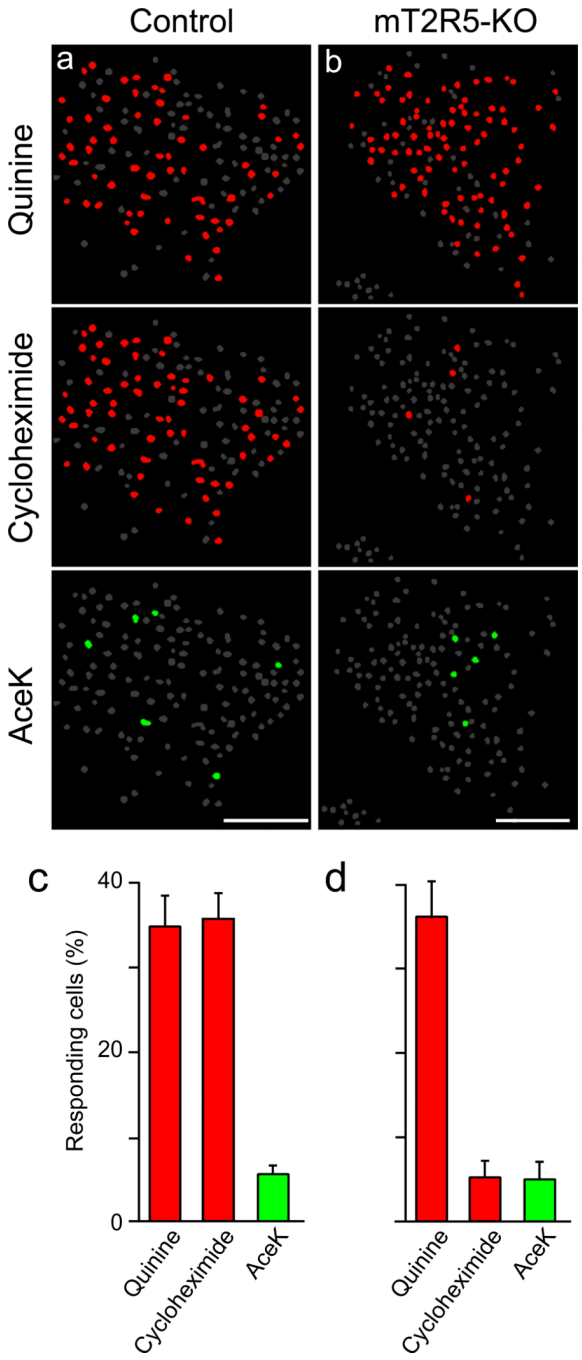

Fig. 3. Responses in the bitter hot spot are dependent on bitter taste receptor function

(a) Different bitter compounds activate the same hot spot in the cortex (see Fig. S3) (n = 4). (b) Animals lacking the bitter taste receptor for cycloheximide (T2R5-KO) selectively lack cortical responses to cycloheximide, but retain normal responses to other bitters (e.g. 10 mM quinine) (n = 2, 7 trials each tastant). (c–d) Quantitation of taste responses in the control and T2R5-KO animals; also shown are data for a sweet tastant (AceK), note the lack of responses in both control and KO animals. Scale (a, b) = 100 μm, error bars are mean ± s.e.m.

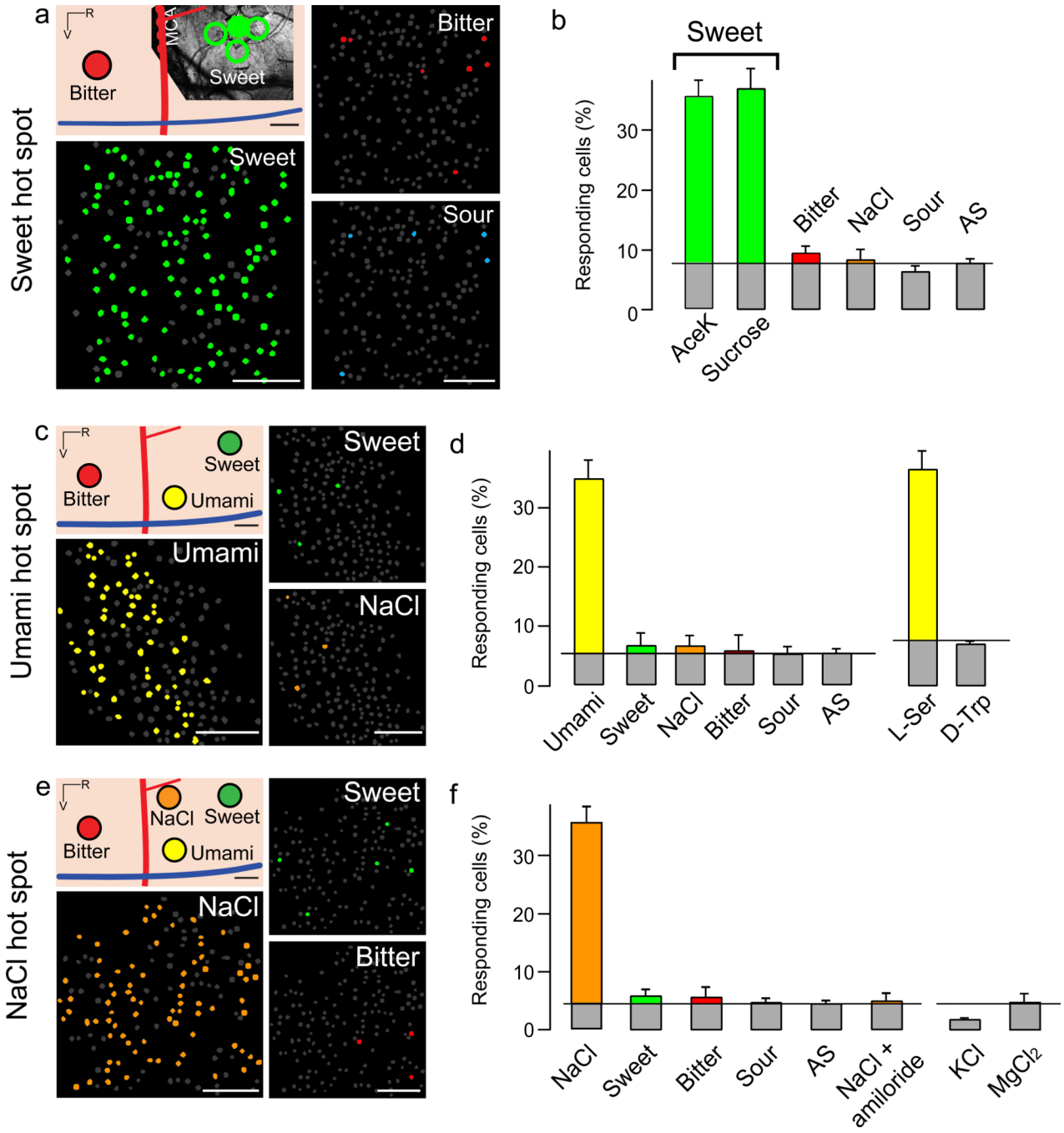

Fig. 4. The basic tastes are represented in a spatial map in primary taste cortex

(a–b) sweet taste is represented in its own cortical field (solid green circle), approximately 2.5 mm rostro-dorsal from the expected location of the bitter hot spot (red circle in upper left illustration). Also shown is the location of the sweet hot spot in 3 additional animals (green open circles). (a) Approximately 35% of the OGB-labeled neurons in the sweet cortical field respond in multiple trials to sweet taste stimulation of the tongue (green-labeled neurons), but not to other tastes (e.g. bitter and sour). (b) The responses are highly specific for sweet tastants (see also Fig. S7), including natural and artificial sweeteners. (c) umami taste is also represented in its own stereotypical hot spot, found approximately 1 mm ventral to the expected sweet cortical field (yellow circle). (d) As anticipated, responses are selective for

umami tastants, including various L-amino acids, but not to D-amino acids or other taste qualities. (e–f) Low concentrations of sodium salt (100 mM NaCl) are known to activate a unique population of sodium sensing taste receptor cells (12), and are represented in a distinct cortical field (orange circle) approximately 1 mm equidistant to the expected sweet and umami hot spots. (f) Importantly, amiloride completely abolishes both the function of the sodium taste receptor, and the insular representation of sodium taste. Other salts are known not to activate the sodium sensor (12), and indeed are not represented in the sodium hot spot (e.g. KCl, $MgCl_2$). See Fig. S5 for additional details on the sweet, umami and sodium responses. Scale bars = 100 μm; cortex diagram= 0.5 mm; error bars are mean ± s.e.m. The hot spots for the different tastes are too far from each other to be imaged on the same animal; reconstructions are based on multiple animals. A minimum of 4 animals, and 4 trials per animal/per tastant were used to define the sweet (n = 10 animals), umami (n = 5 animals) and sodium cortical fields (n = 4 animals; see methods for additional details).